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- a) introducing said embryo into a recipient non-human female of the same species as said recipient oocyte;
 - b) allowing said introduced embryo to develop into a genetically altered non-human mammal; and
 - c) deriving the rejuvenated, genetically modified cell from said mammal.
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136. The method of claim 135, further comprising:

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- a) using the cell having the first and second genetic modifications as a second donor cell;
 - b) transferring said second donor cell, the nucleus of said cell, or chromosomes of said cell, into a second recipient mammalian oocyte of the same species as the donor cell ; and
 - c) generating a re-cloned, rejuvenated, cell which has said first and second genetic modifications.
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151. A method of performing genetic manipulations in mammalian cells, comprising

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- a) bringing a normal, somatic donor mammalian cell to a state of senescence or near senescence;
 - b) transferring the donor cell, the nucleus of said cell, or chromosomes of said cell, into a recipient mammalian oocyte of the same species as the donor cell,
 - c) generating a rejuvenated cell; and
 - d) making a genetic modification in the genome of said rejuvenated cell.
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REMARKS

This Reply is responsive to the Office Action dated August 14, 2002. Entry of the foregoing and reconsideration on the merits pursuant to 37 CFR 1.112 is respectfully requested.

Claims 93-112 and 181-193, directed to a method for producing a rejuvenated cell that does not comprise generating a teratoma, and to products of such a method; and claims 83, 119, 147, 166, and 197, directed to the invention wherein the nuclear donor cell and the recipient oocyte are of different species, are **canceled without prejudice**; the subject matter of these claims will be pursued in a related application.

Regarding the objection to the claims for having internal periods:

Claims 69, 113, 128, 132, 136, and 151 are amended by replacing internal periods with parentheses.

Regarding the rejection of the claims under 35 U.S.C. §112, first paragraph:

Claims 69-231 were rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabled for a method of providing primary cells comprising:

- a) enucleating an oocyte of a first mammalian species and transferring the nucleus of the primary cell into the oocyte of the same species as the donor cell;
 - b) activating the nt unit;
 - c) culturing the activated nt unit in an immunocompromised mouse to produce a teratoma; and
 - d) isolating a differentiated cell from said teratoma, and cells derived from said method,
- is not enabled for methods using any species host cell or for non-mammalian species.

Independent claims 69, 113, 128, 132, 136, and 151 are amended to recite that the donor cell nucleus is transferred into a recipient mammalian oocyte of the same species as the donor cell, and the claims that recited cross-species nuclear transfer are canceled.

The Applicants respectfully traverse the requirement that the claims recite a step of activating a nuclear transfer unit. The specification does not expressly describe a step of activation, because at the time the parent application was filed, methods for cloning by nuclear transfer were well-known in the art - persons skilled in the art knew that activation

was one of the many steps performed in carrying out cloning by nuclear transfer. For example, alternative methods for cloning by nuclear transfer in which the recipient oocyte is activated either before or after transfer of the donor cell nucleus are described in Campbell et al. (Reviews of Reproduction; 1996, 1:40-46, see pages 44-45, attached). A nuclear transfer cloning method in which the recipient oocyte is activated before nuclear transfer is also described in U.S. Patent No. 5,496,720 (see col. 8, line 62, to col. 9, line 8). A nuclear transfer cloning method in which the recipient oocyte is activated by stimulation with an electric field after nuclear transfer is described in Barnes et al. (Mol. Reprod. and Devel., 1993, 36:33-41, see pp. 34-35, attached). Under U.S. Patent Law, it is established that "the specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known to those skilled and already available to the public." See §2164.05, The Manual of Patent Examining Procedure, August 2001, citations omitted. The claims recite transferring a mammalian donor cell that is senescent or near senescence, the nucleus of said cell, or chromosomes of said cell, into a recipient mammalian oocyte of the same species as the donor cell to generate an embryo. The specification clearly describes performing this step as a method of cloning by nuclear transfer (see page 7, line 16, to page 8, line 2). As the above-identified scientific articles show, persons skilled in the art at the time the application was filed knew how to perform cloning by nuclear transfer to generate an embryo, and would have known that activation was a step in that procedure. Accordingly, and given that the specification does not literally describe performing a step of activation, the Applicants respectfully request that the requirement that the claims recite a step of activation be withdrawn.

The Applicants respectfully traverse the requirement that the claims recite a step wherein an activated NT unit is cultured in an immunocompromised mouse to produce a teratoma.

Claims 93-112 and 181-193 that recited a method for producing a rejuvenated cell that does not comprise generating a teratoma are canceled.

Claims 113-127 and 194-209 are drawn to a method of making a cloned mammal, and to products produced by the claimed method; and claims 128-169 and 210-231 are drawn to methods of performing genetic manipulations in mammalian cells, and to products produced by the claimed methods. As described in the specification, for example, at pages 22-25, these embodiments of the invention do not require a step of culturing a cell in an immunocompromised mammal to produce a teratoma. Example 2 demonstrates the operability of the claimed methods of performing genetic manipulations in mammalian cells and of making a cloned mammal having rejuvenated cells. Accordingly, the Applicants respectfully request that the requirement that claims 113-169 and 194-231 recite a step of culturing a cell in an immunocompromised mammal to produce a teratoma be withdrawn.

Claims 69-92 and 170-180 do include a step of culturing a cell in an immunocompromised mammal to produce a teratoma; however, the Applicants traverse the requirement to use an immunocompromised mouse. The specification teaches using any immunocompromised animal that is capable of supporting teratoma formation and is immunocompromised to the extent that it does not reject the developing teratoma (p. 19, lines 5-8). At the time the invention was made, persons skilled in the art were familiar with immunocompromised mammals other than mice that could be used for the claimed invention. For example, Dekel et al. (Transplantation, 1997, 64(11):1550-8) describes SCID rats produced by irradiation; Jezyk et al. (Clin. Immunol. Immunopathol., 1989, 52(2):173-189) describes SCID dogs, and Perryman et al. (J. Protozool., 1991, 38(6):98S-100S) describes SCID horses. Persons skilled in the art would reasonably have expected the claimed invention to operate successfully with any of these. Accordingly, the Applicants respectfully request that the requirement that the claims be limited to methods using immunocompromised mice be withdrawn.

Regarding the rejection of claims under 35 U.S.C. §102(a/e):

Claims 69-231 were rejected under 35 U.S.C. §102(a/e) as being anticipated by Strelchenko (U.S. Patent No. 6,011,197) or Damiani (U.S. Patent No. 6,258,988) as further

evidenced by Evans et al. The rejection states that "the methods as instantly claimed provide no novel steps not disclosed in Strelchenko et al. of Dimiani et al." (sentence bridging pp. 9-10). The premise of the rejection is that the claimed methods and products are inherently the same as those described by Strelchenko et al. of Dimiani et al. The Applicants respectfully traverse the allegation that the claimed methods and products are indistinguishable from those described by Strelchenko et al. of Dimiani et al.

The claims all recite transferring a senescent or near-senescent donor mammalian cell, or the nuclei or chromosomes of said cell, into a mammalian oocyte, and generating an embryo, fetus, or animal, the cells of which are rejuvenated relative to age-matched control cells, as evidenced by increased proliferation life-span, telomere length, and activities of EPC-1 and telomerase. Strelchenko et al. describes re-programming non-totipotent primordial germ cells into totipotent embryonic germ cells (col. 2, line 54), and using bovine fibroblast cells as donor cells in multiple nuclear transfer cycles (col. 50, lines 1-6); and Damiani similarly suggests using porcine fibroblast cells as donor cells in multiple nuclear transfer cycles (col. 49, lines 22-29). However, none of the three cited references disclose or suggest using cells that are senescent or near senescence as donor cells for nuclear transfer, nor do the cited references disclose or suggest that the claimed method may be used to generate rejuvenated cells with restored or lengthened telomeres and increased proliferation life-span. In fact, the prior art taught away from practicing the claimed invention. As discussed in the specification and in the reply to the previous Office Action, and acknowledged by the Examiner in the Office Action, Shiels et al. (Nature (1999) 399: 316) taught away from using senescent or near senescence as donor cells for nuclear transfer, by teaching that the telomeres of a sheep cloned by nuclear transfer are just as short as those in the donor cell. These findings suggested to those skilled in the art that cells created by nuclear transfer might undergo premature senescence, and that cloned animals generated by nuclear transfer could exhibit decreased life spans. Prior to the present invention, known nuclear transfer techniques were thought to result in cloned cells, embryos, animals, etc. having telomeres of the same lengths as those of the donor cell used to create them, so that

one skilled in the art would attempt to minimize the number of replications a cell would undergo before using it as a donor cell in nuclear transfer. The present invention goes directly against this view - it shows that cells that can be cultured extensively, as occurs when carrying out complex genetic modifications, even to the point that the cells are senescent or near senescence. Moreover, the telomeres that are degraded during extensive culture of somatic cells are restored and rejuvenated "hyper-young" cells with increased proliferative life-span are generated when such senescent or near senescent cells are used as donor cells for nuclear transplant. Nothing in the prior art suggested this surprising and important result.

Neither Strelchenko nor Damiani teach that a cell's genetic lifespan, related to the lengths of the telomeres, could be rejuvenated using nuclear transfer. In fact, the methods disclosed in Strelchenko employ the same type of donor cell used to create Dolly, i.e., a quiescent cell (see the paragraph bridging columns 11 and 12). Quiescent cells are cells that have been serum-starved, and are different than cells that have naturally aged. Furthermore, the use of quiescent cells as nuclear donors results in cloned cells that do not have regenerated telomeres (as evidenced by Dolly). The cited prior art references simply do not disclose, either directly or inherently, the claimed methods of nuclear transfer using senescent or near senescent donor cells to produce rejuvenated primary cells, i.e., cells that have telomeres that are as long as, or longer than, those of age-matched non-cloned cells.

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." See §2131, The Manual of Patent Examining Procedure, August 2001, citations omitted. Neither Strelchenko et al., Dimiani et al., nor Evans et al. disclose or suggest cloning by nuclear transfer using a senescent or near-senescent donor cell, nor do they disclose or suggest that such a method would result in production of rejuvenated cells as defined and described in the specification; i.e., cells with restored or lengthened telomeres and increased proliferation life-span. Accordingly, the Applicants respectfully request that the rejection of the claims under §102 (a/e) be reconsidered and withdrawn.

Regarding the rejection of claims under 35 U.S.C. §102(b):

Claims 69-231 were rejected under 35 U.S.C. §102(b) as being anticipated by Robl (WO 98/07841) as evidenced by Evans. Again, the basis for the rejection is that the claimed methods do not differ from those disclosed in Robl and Evans. The Robl reference does not disclose, either directly or inherently, a method of cloning by nuclear transfer that uses a nuclear donor cell that is senescent or near-senescent, nor does it disclose or suggest the surprising finding that such a method results in production of rejuvenated cells. The specification clearly describes and defines the rejuvenated cells produced by the claimed method as having restored or lengthened telomeres and increased proliferation life-span. Therefore, the Applicants respectfully request that the §102(b) rejection based on Robl be reconsidered and withdrawn.

Regarding Rejection of the Claims for Provisional Double Patenting:

Claims 69-231 of the present application are provisionally rejected under 35 U.S.C. § 101 as claiming the same invention as the claims of co-pending Application Nos. 09/520,879 and 09/656,173. (Application No. 09/520,879 is incorrectly identified as 09/250879 in the Office Action). Applicants respectfully request that this rejection be held in abeyance until allowance is negotiated. At that time, if the claims in the instant application are still found to claim the same invention as claims of the co-pending Applications, Applicants will submit a terminal disclaimer to obviate this rejection.

The Applicants' affirm that a terminal disclaimer will be submitted when the claims in the instant application are found to be allowable, but for the outstanding double patenting rejection over claims of co-pending Application Nos. 09/520,879 and 09/656,173. If additional response to the provisional double patenting rejection is required, or if the Examiner has any further questions or issues to raise regarding the subject application, it is respectfully requested that he contact the undersigned so that such issues may be addressed expeditiously.

All issues raised by the Office Action dated August 14, 2002, have been addressed in this Reply. It is respectfully submitted that the present application is in a condition for allowance and a Notice to that effect is earnestly solicited. If the Examiner has any further questions or issues to raise regarding the subject application, it is respectfully requested that he contact the undersigned so that such issues may be addressed expeditiously.

Respectfully submitted,

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Date: November 14, 2002

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Enclosures

APPENDIX

The claims are amended as shown below:

69. A method for producing a rejuvenated cell, comprising

- a[.] transferring a mammalian donor cell that is senescent or near senescence, the nucleus of said cell, or chromosomes of said cell, into a recipient mammalian oocyte of the same species as the donor cell to generate an embryo;
- b[.] obtaining an embryonic disc cell, an inner cell mass cell, or an embryonic stem cell using said embryo;
- [b.]c) injecting said embryonic disc cell, inner cell mass cell, or embryonic stem cell into an immune-compromised mammal to form a teratoma;
- [c.]d) isolating the resulting teratoma;
- [d.]e) identifying specific cell types of said teratoma; and
- [e.]f) isolating a rejuvenated mammalian cell from the teratoma.

113. A method of making a cloned mammal comprising rejuvenated cells, comprising:

- a[.] transferring a mammalian donor primary cell that is senescent or near senescence, the nucleus of said cell, or chromosomes of said cell, into a recipient mammalian oocyte of the same species as the donor cell to generate an embryo;
- b[.] introducing said embryo into a recipient non-human female of the same species as said recipient oocyte; and
- c[.] allowing said introduced embryo to develop into a non-human mammal.

128. A method of performing genetic manipulations in mammalian cells, comprising:

- a[.]) making a genetic modification in the genome of a mammalian primary donor cell;
- b[.]) bringing the donor cell to a state of senescence or near senescence;
- c[.]) transferring the genetically modified donor cell, the nucleus of said cell, or chromosomes of said cell, into a recipient mammalian oocyte of the same species as the donor cell to generate an embryo, and
- d[.]) generating a rejuvenated, genetically modified cell from said embryo.

132. The method of claim 128, wherein the donor cell is a non-human cell, and the step of generating a rejuvenated, genetically modified cell comprises:

- a[.]) introducing said embryo into a recipient non-human female of the same species as said recipient oocyte;
- b[.]) allowing said introduced embryo to develop into a genetically altered non-human mammal; and
- c[.]) deriving the rejuvenated, genetically modified cell from said mammal.

136. The method of claim 135, further comprising:

- a[.]) using the cell having the first and second genetic modifications as a second donor cell;
- b[.]) transferring said second donor cell, the nucleus of said cell, or chromosomes of said cell, into a second recipient mammalian oocyte of the same species as the donor cell ; and

c[.]) generating a re-cloned, rejuvenated, cell which has said first and second genetic modifications.

151. A method of performing genetic manipulations in mammalian cells, comprising

a[.]) bringing a normal, somatic donor mammalian cell to a state of senescence or near senescence;

b[.]) transferring the donor cell, the nucleus of said cell, or chromosomes of said cell, into a recipient mammalian oocyte of the same species as the donor cell,

c[.]) generating a rejuvenated cell; and

d[.]) making a genetic modification in the genome of said rejuvenated cell.